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Epizootics in Yellowtail Flounder, *Limanda ferruginea* Storer, in the Western North Atlantic Caused by *Ichthyophonus*, an Ubiquitous Parasitic Fungus

George D. Ruggieri, S.J.,[1] Ross F. Nigrelli,[1] P. M. Powles,[2] and D. G. Garnett[3]

(Plates I-X; Text-figure 1)

Yellowtail flounders (*Limanda ferruginea*) were collected from several areas off Nova Scotia and analyzed for *Ichthyophonus*. The infection was confined to flounders from the Sable Island Bank and Western Bank. Extensive lesions caused by the fungus were present in the heart, liver, kidney, spleen, gastrointestinal tract, and body musculature. The gills, gall bladder, brain, and testes were mildly infected. All developmental stages of the fungus were observed, although in most histological sections the fungi appear as "resting" or as stages in germination and hyphal development. An analysis of infected fish indicates no relationship to sex and that yellowtails in the size range from 24 cm to 40 cm are more heavily infected. The pathological manifestations of the fungus in the various organs and their significance are discussed.

Introduction

Ichthyophonus, the cause of systemic mycosis in fishes, is characterized macroscopically by the presence of single, multiple, or confluent whitish cyst-like lesions in the viscera and in other parts of the body. The disease has been reported in a wide variety of feral and captive fishes of fresh water, brackish water, and marine habitats in cold, temperate, and tropical parts of the world. In every case the causative organism has been identified as a single, pleomorphic species, *Ichthyophonus hoferi* Plehn and Mulsow, 1911. Whether or not a single species of fungus is indeed responsible for the disease in all host species remains to be established. This fungus had also been referred to as *Ichthyosporidium hoferi* (Plehn and Mulsow) Pettit, 1911. The generic name *Ichthyosporidium* Caullery and Mesnil, 1905, is now restricted to certain protozoan parasites of fishes and marine invertebrates in the order Haplosporidia, class Sporozoa (Sprague, 1965).

Most of the literature on *Ichthyophonus* deals with sporadic cases. However, recurring incidences in epizootic proportions have been reported in: the sea herring (*Clupea harengus*) in the western North Atlantic (see Sindermann, 1961, 1966, and 1970 for reviews); the mackerel (*Scomber scomber*) in the eastern North Atlantic (Sproston, 1944); rainbow trout (*Salmo gairdneri*) in hatcheries in the western United States (Rucker and Gustafson, 1953); and, more recently, yellowtail flounder (*Limanda ferruginea*) in the western North Atlantic, especially from western Sable Island Bank (Powles, Garnett, Ruggieri and Nigrelli, 1968).

The present investigation deals with the pathology of the disease in the yellowtails. It also includes a further analysis of the distribution of the infection in fish collected in 1967 from several areas off Nova Scotia, including the site where the epizootic first occurred in 1966.

Material and Methods

The yellowtail flounders were collected from the Gulf of St. Lawrence, Banquereau, Middle Ground, Western Bank, and Sable Island Bank (text-fig. 1.) The sampling routine was as follows: 200 flounders were collected from each area; from each group a sub-sample of 50 specimens preserved in formalin was shipped to the Pathology Laboratory of the Osborn Laboratories of Marine Sciences, New York Aquarium, for detailed microscopic examination of the infection. All fish were examined and the organs of those showing macroscopic evidence of the lesions

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were embedded in paraffin, sectioned between 3 and 5 microns, and stained with Harris' hematoxylin-eosin, Periodic acid Schiff, Bauer's chromic acid Schiff, Gram Weigert, Mallory phosphotungstic acid hematoxylin, Masson's trichrome stain, and Mallory's modification of the Azan staining method.

Observations

1. *Incidence and distribution.* Text-figure 1 is a map showing the localities from which the samples were taken. The infection was confined to the Sable Island Bank and the Western Bank, with incidences of 25 percent and 57.4 percent, respectively. The incidence seems to decrease eastward; e.g. yellowtails from south of Sable Island show only a 2.8 percent incidence. Fish from all other areas, including the Gulf of St. Lawrence, appeared to be free of the infection, at least macroscopically. The evidence indicates that the stocks from Sable Island, Banquereau, and St. Lawrence do not mix. It is suggested that the isolation of the disease to the Sable Island area may be related to higher bottom temperatures. Whether or not this is a significant factor remains to be determined.

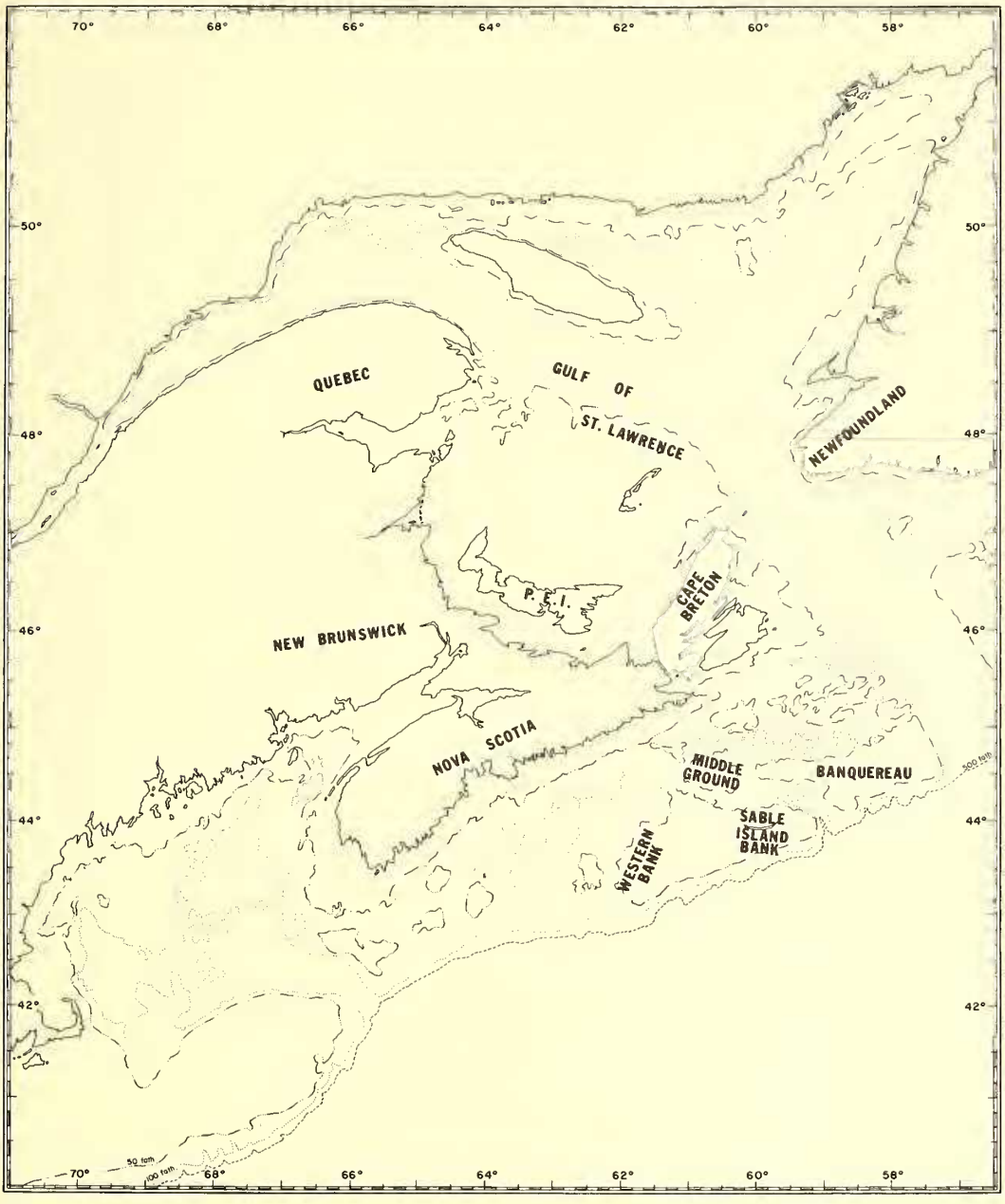
An analysis (Table 1) of the fish collected from Western Bank and Sable Island Bank shows the incidence of the disease in relation to size and to the intensity of the infection as seen macroscopically in the liver, heart, kidney, and gastro-intestinal tract.

There is no apparent relationship as to sex, although there is some evidence that males appear to be more susceptible. Yellowtails in the size range from 24 cm to 40 cm are more susceptible; the disease appears to be present in proportion to the most abundant sizes, which fits in with the observation that there is little or no size segregation in this species. Further, a preliminary analysis (Garnett and Powles) suggests that there is no significant length-weight (coefficient of condition) relationship between diseased and normal fish. A growth and mortality study should be done to make such observations significant. It should be emphasized that the absence of obvious lesions in both younger and older yellowtails as shown in Table 1 does not indicate that they are entirely free of the fungi, since it is possible that deep-seated and isolated "cysts" may be present, e.g. in the brain.

2. *Parasite.* Because of certain morphological and pathological characteristics, the causative organism was identified as a phycomycete of the genus *Ichthyophonus.* Whether or not *Ichthyophonus hoferi* is the specific agent responsible for the mycosis in the yellowtails can only be determined after a more thorough study of the organism under cultural conditions, by experimental infections, and by comparative analysis with isolates from other freshwater and marine hosts. The use as a diagnostic procedure of measurements and descriptive terms for the various stages seen in tissue sections is at present meaningless. All stages in the development are identifiable, but in most sections the fungi appear as "resting," often thick-walled cysts (e.g., Pl. III, fig. 6), or as stages in germination and hyphal development (Pl. III, fig. 5, and Pl. VII, fig. 13) generally characteristic of this type of mycotic infection in fishes. The presence of condia-like bodies in kidney tissue (P1. V, fig. 10) is interesting; similar spores have been reported by Sproston (1944) in fungal cultures made from mackerel and their development was also referred to by Reichenbach-Klinke and Elkan (1965) for *Ichthyophonus* infection in other marine fishes, but not the herring.

3. *Pathology.* Extensive lesions caused by the fungus were present in the liver (P1. I-III, figs. 2-6), kidney (Pl. IV-V, figs. 7-10), spleen (Pl. VI, fig. 11), body musculature (Pl. VI, fig. 12; Pl. VII, fig. 13), heart Pl. I, fig. 1; Pl. VII, fig. 14; and Pl. VIII, fig. 15), gastro-intestinal tract (P1. VIII-X, figs. 16-19) and to a lesser degree in the gills, gall bladder, brain, and testes (Pl. X, fig. 20). Lesions were not present in the ovary. The absence of the infection in the ovary and the relatively mild pathology in the testes indicates that the disease may have very little effect on potential reproductive ability.

As is well known for mycotic diseases, no one tissue change is entirely pathognomic of the fungus infection in fish. In the yellowtails, the lesions are generally characterized by the absence of classical inflammatory responses. However, the lesions involve a great deal of necrosis, especially in those areas showing activities associated with germination and hyphal growth. In areas where numerous "resting cysts" are present, the fungi are relatively inert and behave as foreign bodies, i.e. they become surrounded by histiocytes (epithelioid elements), typical of many granulomas (P1. V, fig. 9; P1. VI, fig. 11; and P1. VIII, fig. 16), or by connective tissue fibers (P1. II, figs. 3, 4; P1. IV, fig. 7; P1. VII, fig. 14; P1. VIII, fig. 15; P1. X, fig. 20). This is not surprising since similar reactions have been reported for certain mycoses in humans and other mammals. In relatively heavy infections, atrophy effects due to pressure with concomitant necrosis are quite evident in the parenchymal tissue, e.g. liver (P1. II, figs. 3, 4) and kidney (P1. IV, figs. 7, 8, and P1. V, fig. 9), and in the heart (P1. VII, fig. 14, and P1. VIII, fig. 15) and body musculature (P1. VI, fig. 12).



TEXT-FIGURE 1. Map of Western North Atlantic. Yellowtail flounders were collected from the Gulf of St. Lawrence, Banquereau, Middle Ground, Western Bank, and Sable Island Bank.

TABLE 1. MACROSCOPIC ANALYSIS OF FISH FROM WESTERN BANK AND SABLE ISLAND BANK.

Total length in cm	*Total number fish*	*Number Infected*	*Intensity of Infection*			
			Liver	*Heart*	*Stomach Intestine*	*Kidney*
16	4	0				
17	3	0				
18	2	0				
19	2	0				
20	5	0				
21	3	0				
22	5	1	+			
23	2	0				
24	2	1	++++		+	
25	2	1	+++	+++	+	
26	7	3	++++	++	+	++++
			+++	+++	+++	+++
			++	++	+	++
27	7	1	++++	+++		
28	6	2	++++		+++	+++
			++	+++		+++
29	4	3	+++	+++	+	+
			+++	+++		++
			++	+++	+	++
30	8	5	+	+		
			++++	+++	+	+++
			+	++		
			++++	++++	++++	++++
			++++		+++	++++
31	6	1	+		+	+
32	3	2	+++		+	+
			++	++		
33	8	5	++++	++++	++++	++++
			++++	++++		
			+++	++	+	+++
			++	+	+	+
			+		+	
34	3	1	++	+++		
35	5	4	+++	+++	+++	+++
			+++	+++	+	++
			+++	+++	+	+
			+			
36	4	2	++++	++++	+	++
			+			+
37	5	3	++++	+++	++	+++
			+++	+++	+++	+++
			++	+++	+	+
38	1	0				
39	1	1	++			
40	2	2	+++	+++		+++
			+			
41	1	0				
43	1	1	+++			
44	2	0				
46	2	1	+	+++		

\+ = less than 5 small cyst-like lesions
++ = 6-10 small cyst-like lesions
+++ = 10-20 small cyst-like lesions or 1 large confluent patch
++++ = over 20 small cyst-like lesions or several large confluent patches

Discussion and Summary

The yellowtail flounder *(Limanda ferruginea)* is the third major species of North Atlantic food fish to be affected by recurring epizootics caused by the fungal parasite *Ichthyophonus,* with incidences ranging from 2.8 percent to 57.4 percent. The other two species, referred to above, are the Atlantic herring and mackerel, with incidences ranging from 2-80 percent (average 25 percent) and 38-70 percent respectively (Walford, 1958). The disease in the yellowtails appears to be limited to populations in the area of Sable Island off the coast of Nova Scotia but the effects of the infection on growth and on mortality rate are at present unknown. The absence of striking evidence of mass mortalities or fluctuations in the populations of this species in the epizootic regions is surprising. The damage to such vital organs as the heart, liver and kidney is so extensive that there can be no question that homeostasis is affected to the extent that many must succumb directly to the infection, or are made so weak that they become easy prey, or are readily killed off by any drastic change in the physical and chemical characteristics of the environment.

The absence of the disease in yellowtails in the Gulf of St. Lawrence is puzzling since this is one of the areas in which epizootics in the herring have been reported almost in a cyclic fashion since 1900 (Sindermann, 1970).

Acknowledgments

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EXPLANATION OF PLATES

Plate I

Fig. 1. Gross manifestation of lesions on the heart of a yellowtail caused by the phycomycete *Ichthyophonus.*

Fig. 2. Numerous cysts of the parasite in the liver.

Plate II

Fig. 3. Liver showing numerous "resting cysts." Note extensive damage and development of connective tissue. Azan.

Fig. 4. Area of the liver showing necrosis and distortion of the parenchymal architecture. Azan.

Plate III

Fig. 5. Germination and hyphal development in liver with extensive necrosis of the parenchyma. Note absence of typical inflammatory reaction. Resting cysts strongly PAS positive.

Fig. 6. A typical resting cyst with multi-nucleated plasmodium in the liver. Hematoxylin-eosin.

Plate IV

Fig. 7. Extensive infection in the kidney with damaged tubular elements. Bauer's.

Fig. 8. Area of infected kidney with extensive necrosis. Gram Weigert.

Plate V

Fig. 9. Granuloma-like reaction around a "resting cyst" in the kidney. Note the massing of histiocytes and degenerative changes of tubules due to pressure effects. Azan.

Fig. 10. Conidial elements of *Ichthyophonus* seen in the kidney. Cysts surrounded by histiocytes. Hematoxylin-eosin.

Plate VI

Fig. 11. Granulomatous reaction around "resting cysts" in the spleen. Masson's.

Fig. 12. Infection in the body musculature showing damage characteristic of Zenker's degeneration. Mallory's.

Plate VII

Fig. 13. Details of stages of germination in the muscle pathway. Hematoxylin-eosin.

Fig. 14. Myocardial degeneration caused by the fungal infection. Note extensive connective tissue development resulting in fibroid swelling. Azan.

Plate VIII

Fig. 15. Germination of fungus in myocardium causing necrosis of the heart muscle fibers; granulomatous lesions were also seen in the pericardium. Hematoxylin-eosin.

Fig. 16. Submucosa of the stomach showing the pressure effects of the parasite and the massing histiocytes on the mucosa. Masson.

Plate IX

Fig. 17. Nest of fungal elements in the submucosa of the large intestine; the small intestine was equally infected. Hematoxylin-eosin.

Fig. 18. Cysts in the mucosa of the large intestine. The basement membrane has been penetrated. PAS.

Plate X

Fig. 19. Multi-nucleated cysts in a capillary of the submucosa of the stomach. Hematoxylin-eosin.

Fig. 20. A fungal cyst in the testis. No pathological changes were noted in spermatogonia or spermatids. Hematoxylin-eosin.

FIG. 1

FIG. 2

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

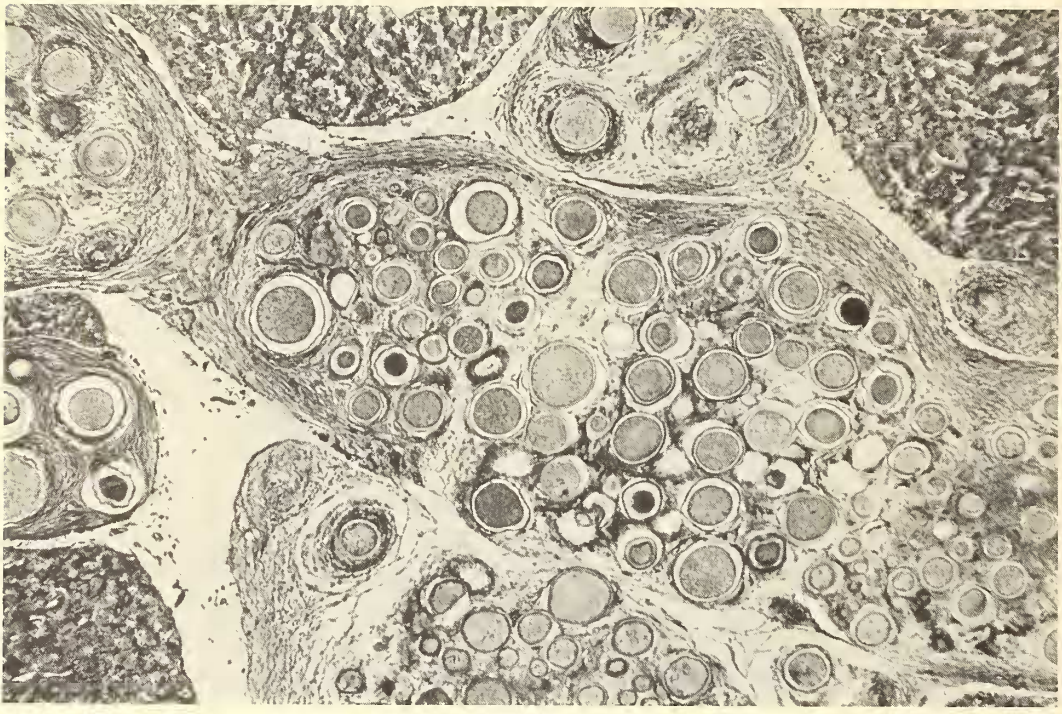


FIG. 3

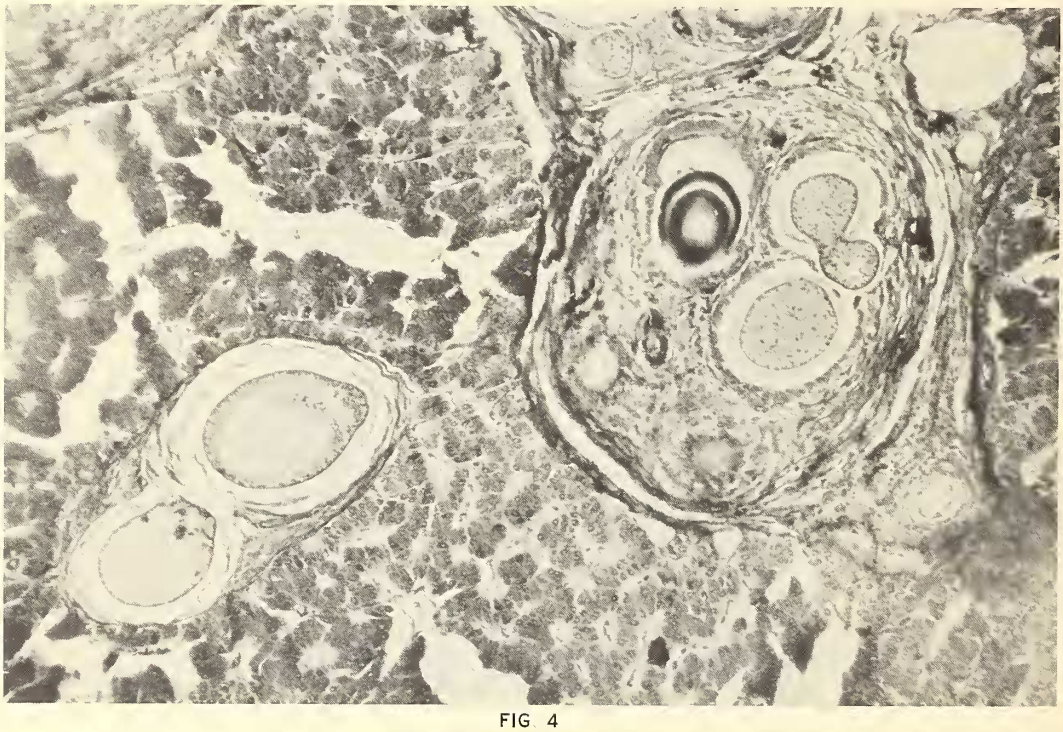
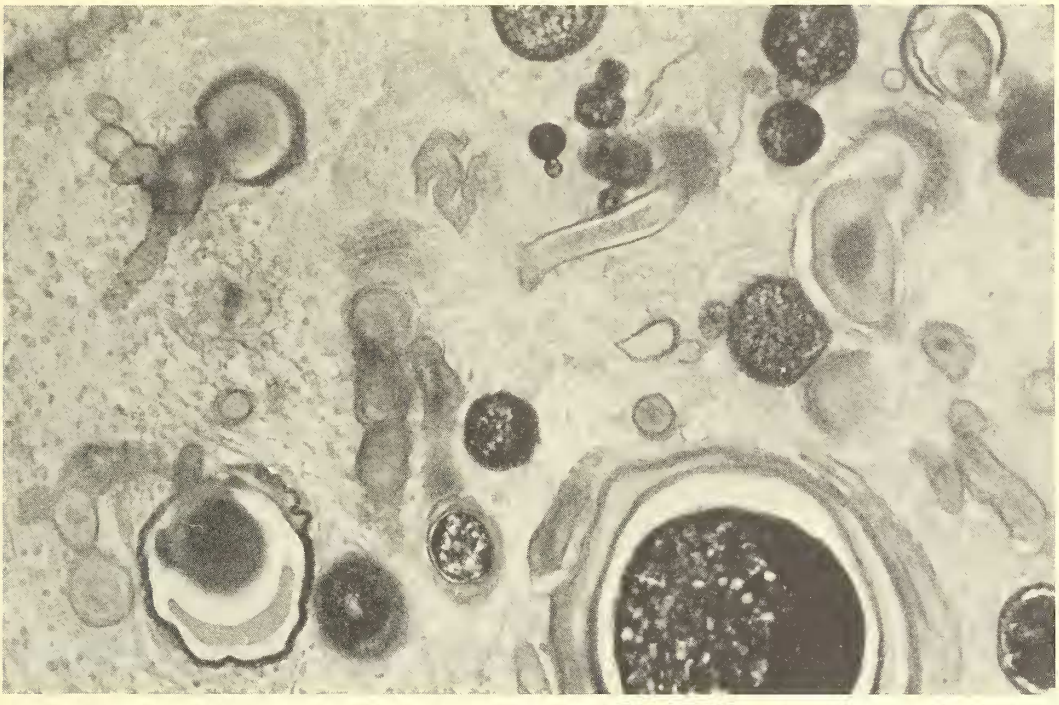


FIG. 4

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS



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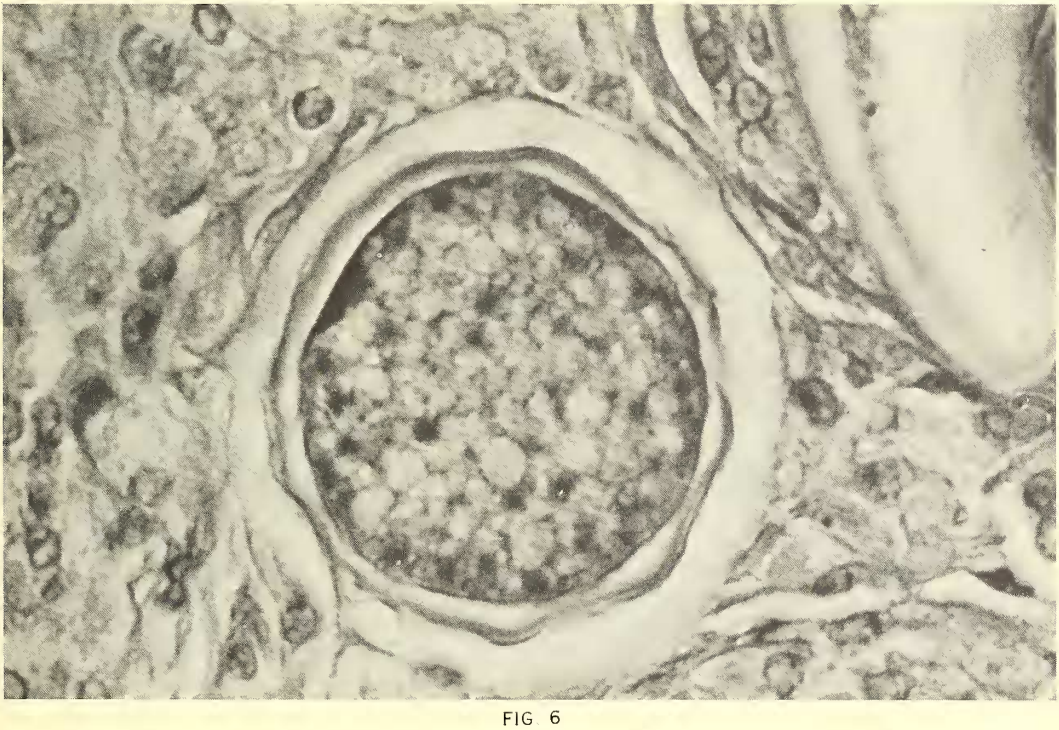

FIG. 6

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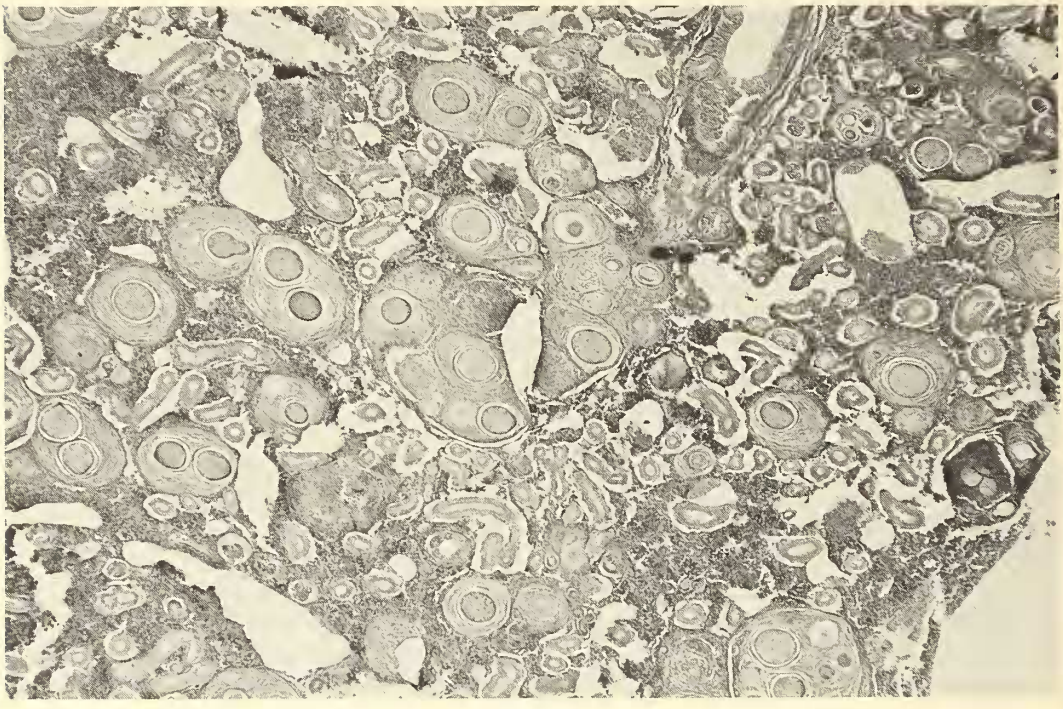

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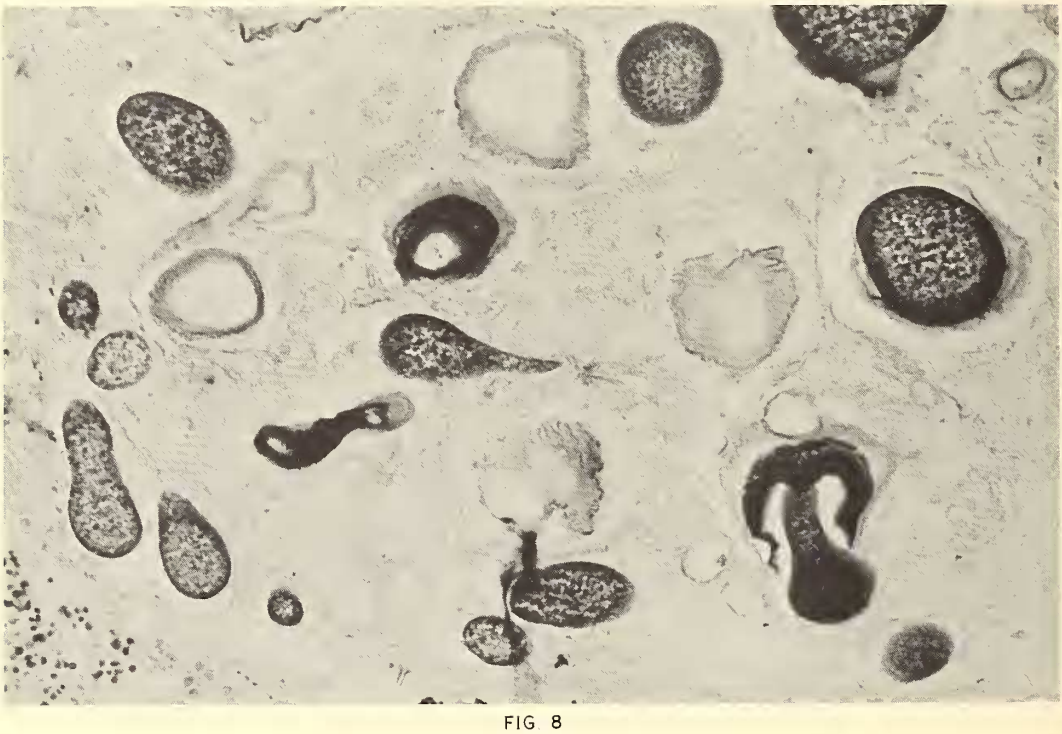

FIG. 8

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

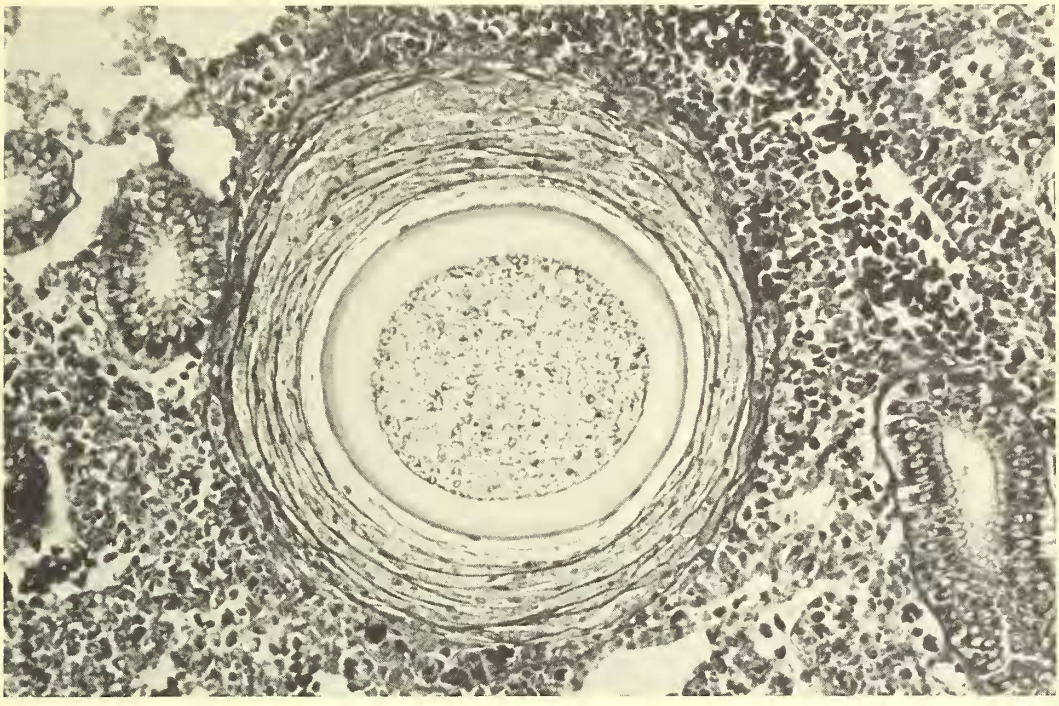

FIG. 9

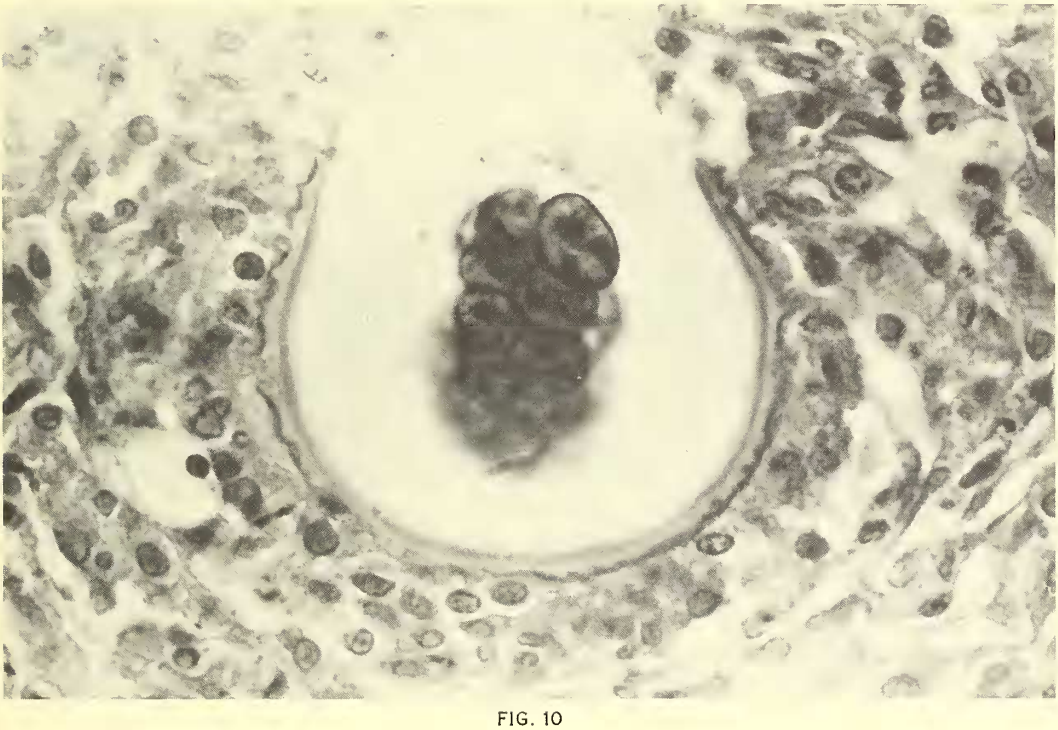

FIG. 10

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

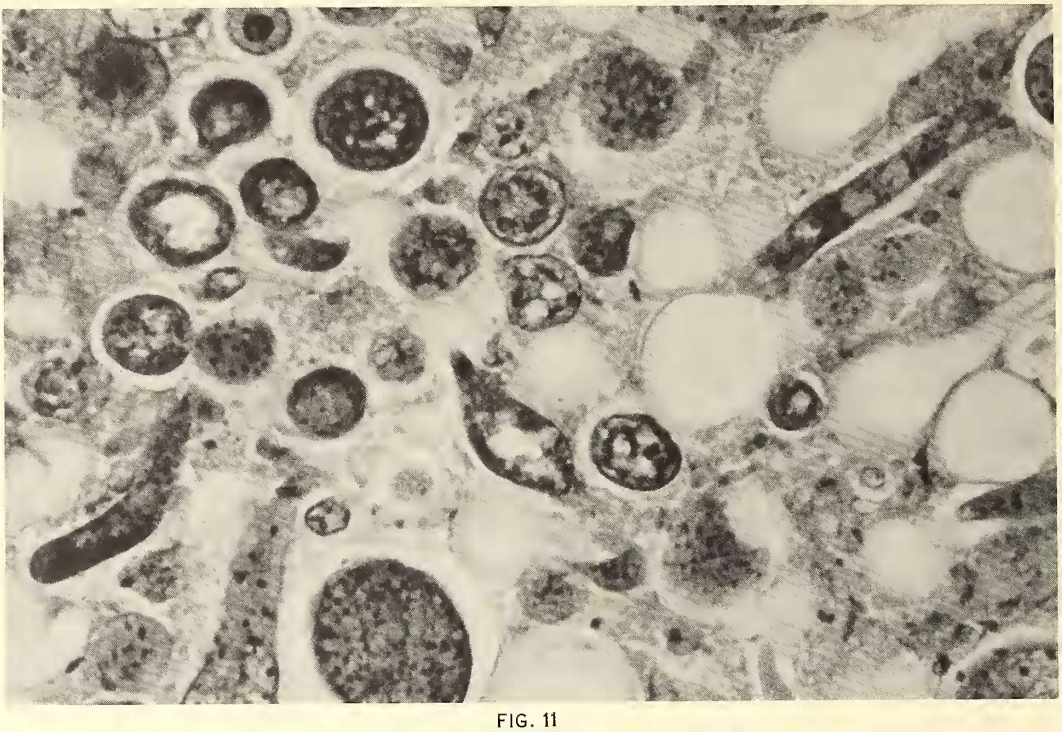

FIG. 11

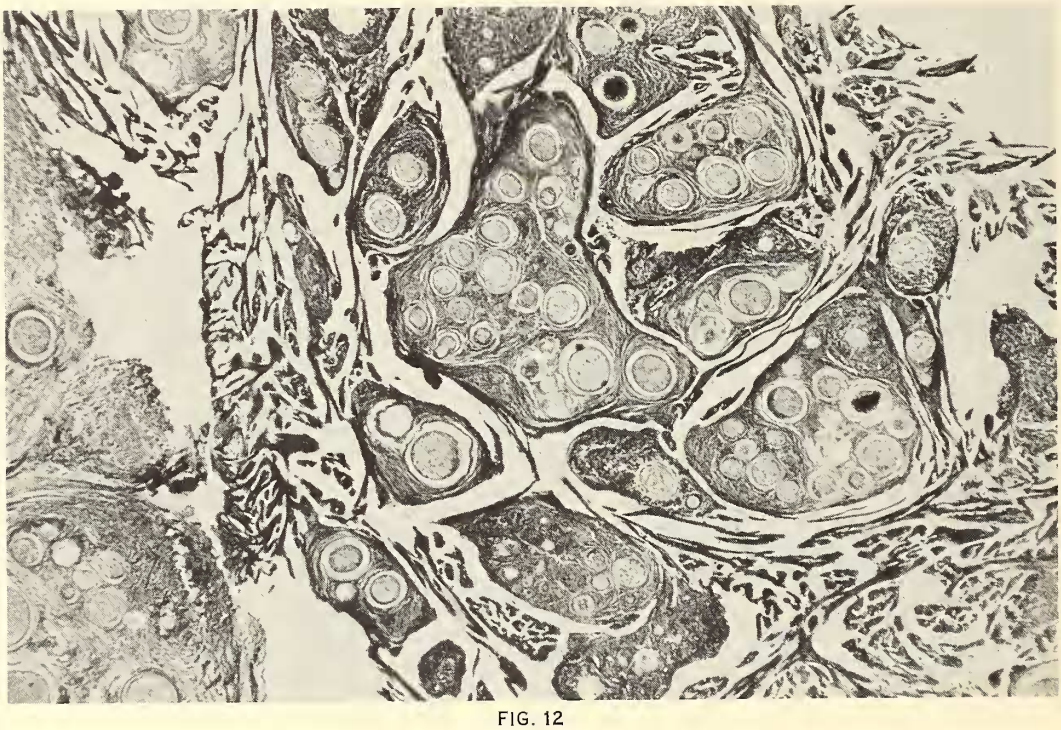

FIG. 12

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

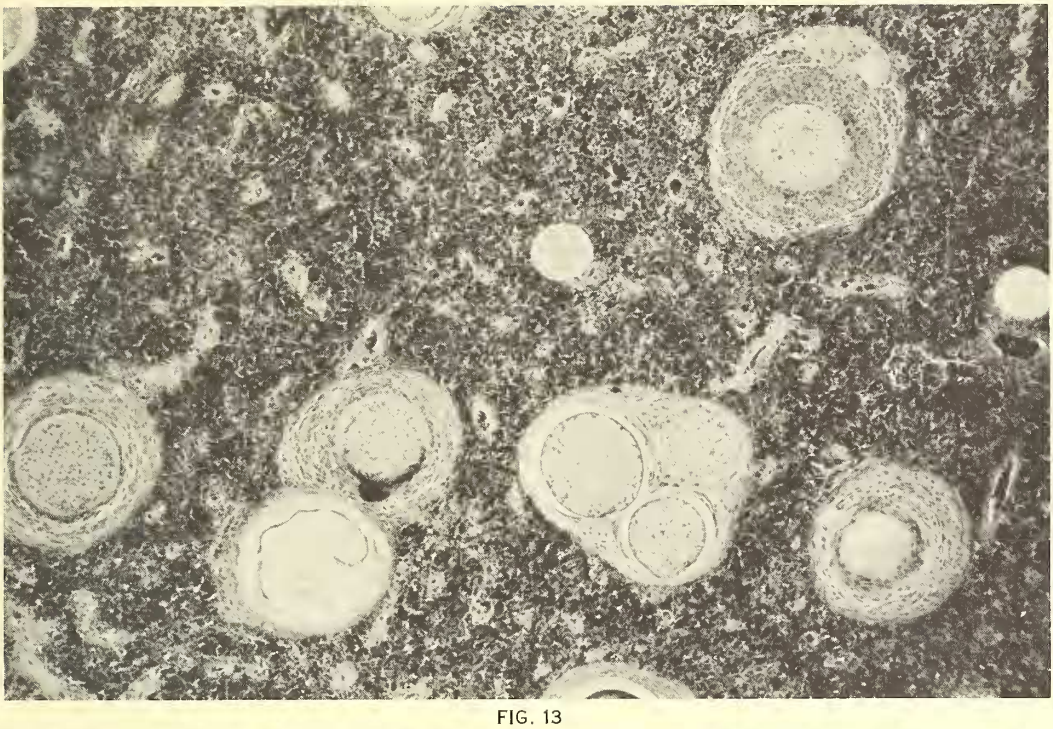

FIG. 13

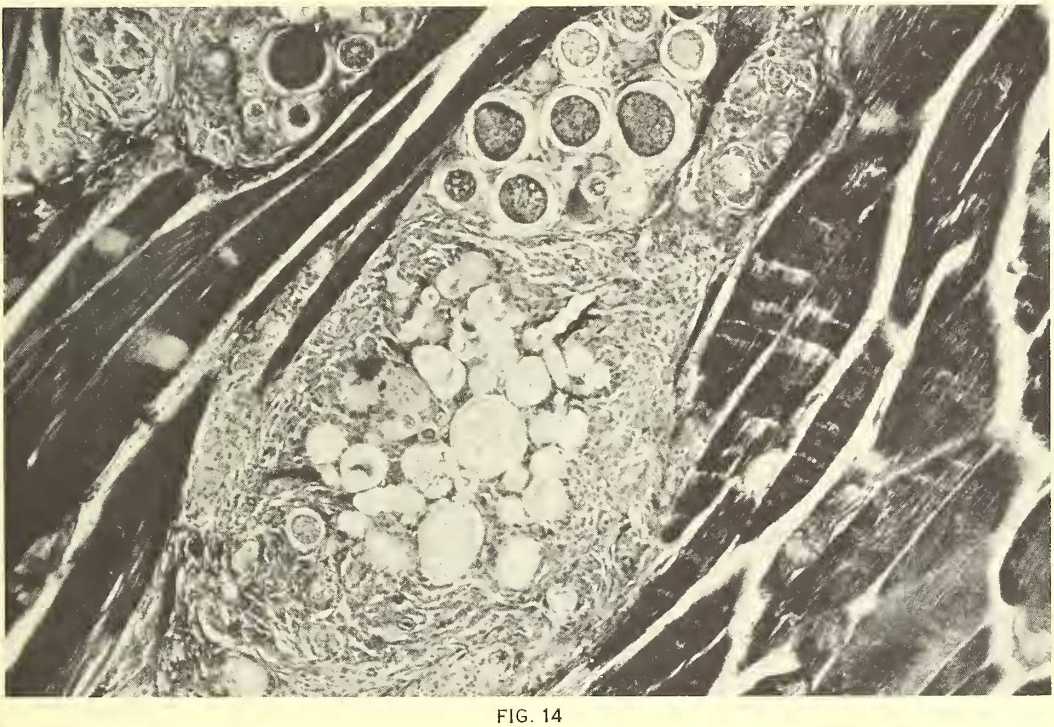

FIG. 14

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

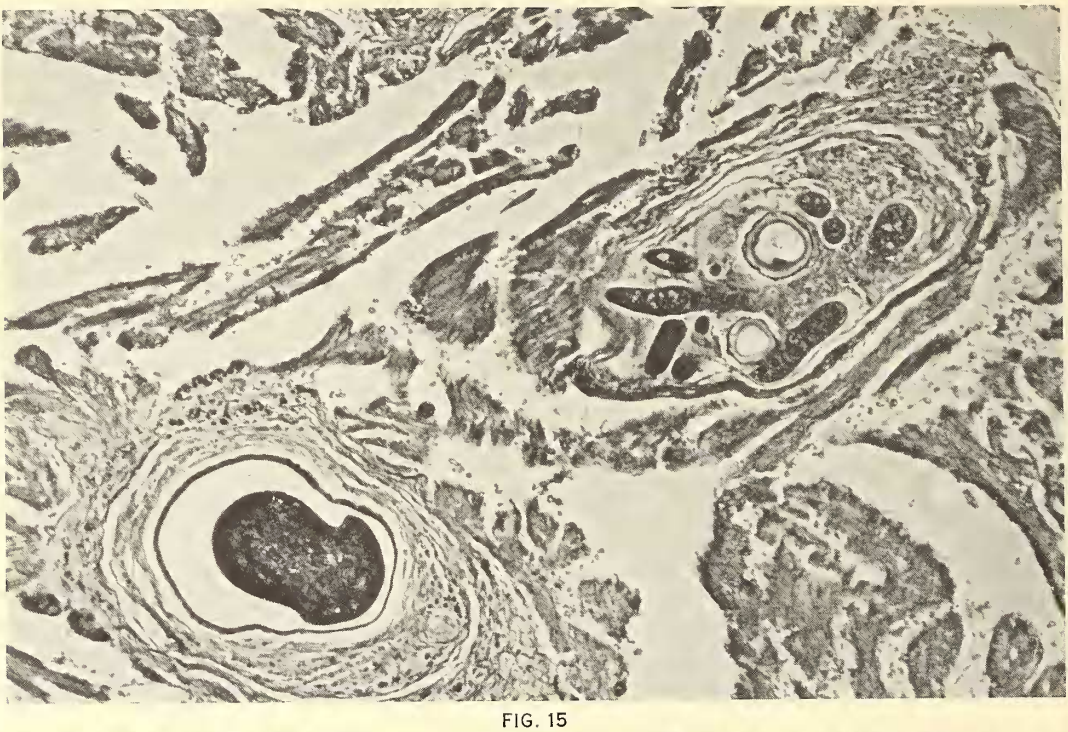

FIG. 15

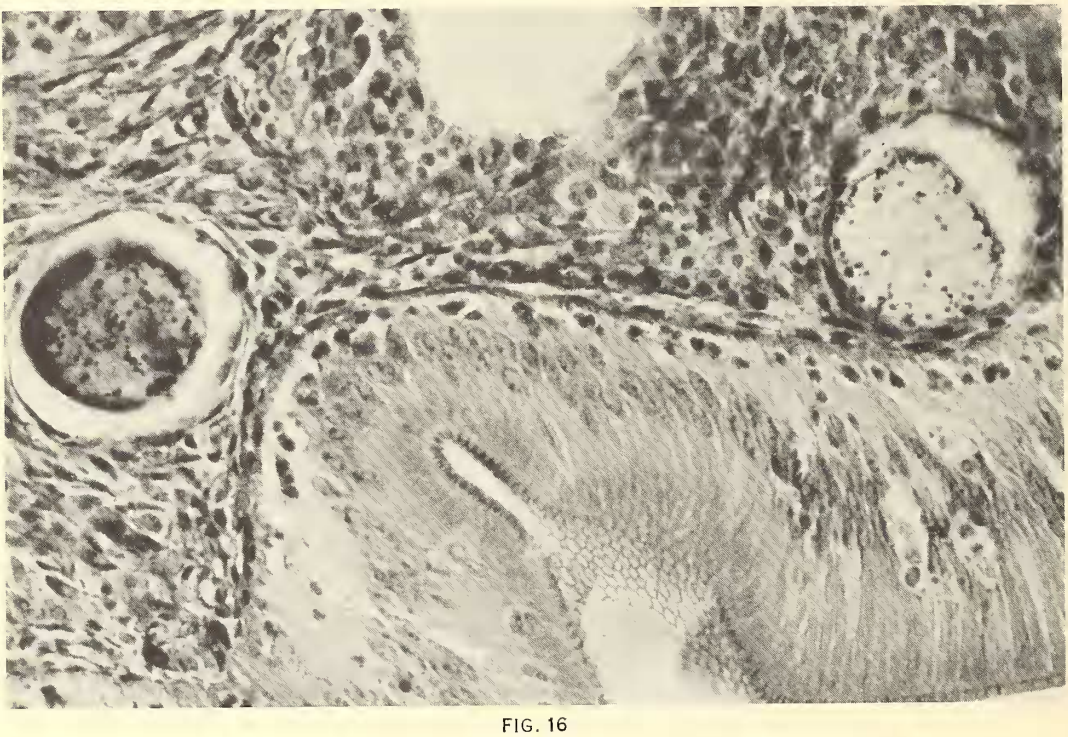

FIG. 16

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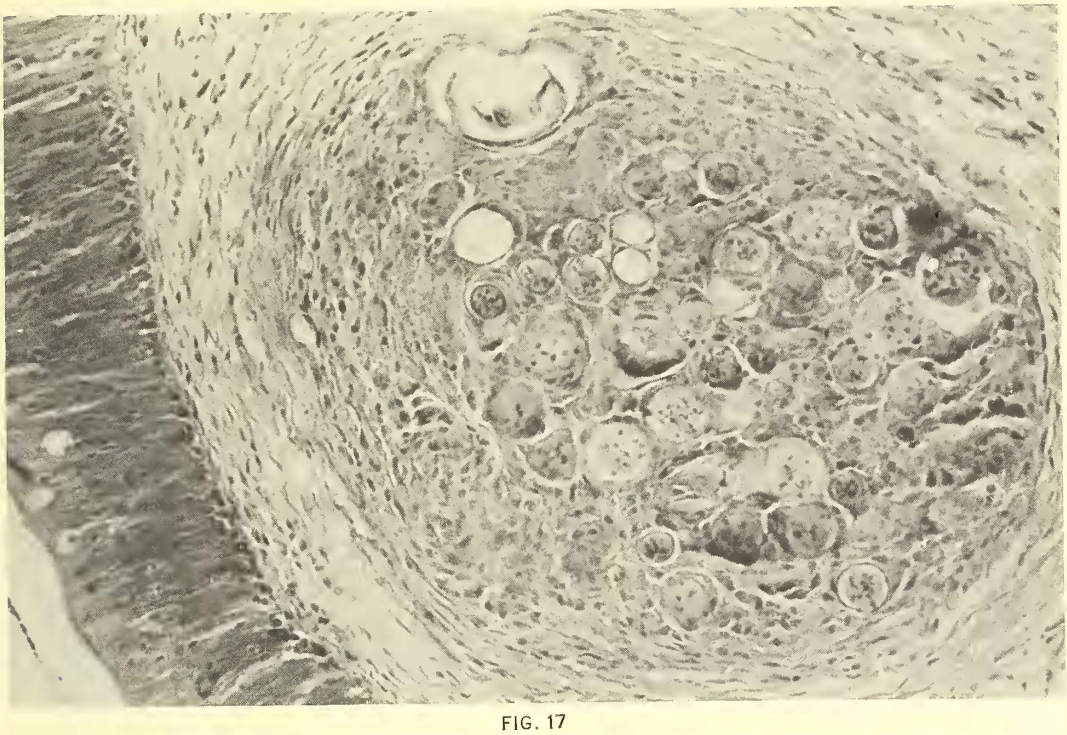

FIG. 17

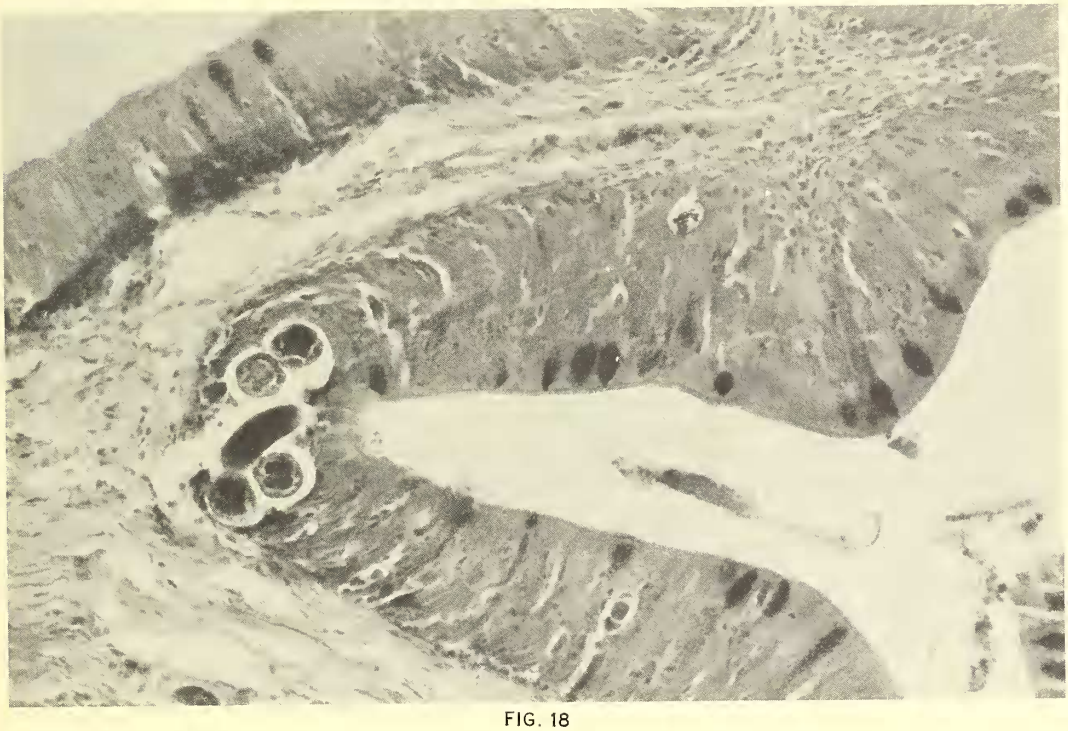

FIG. 18

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

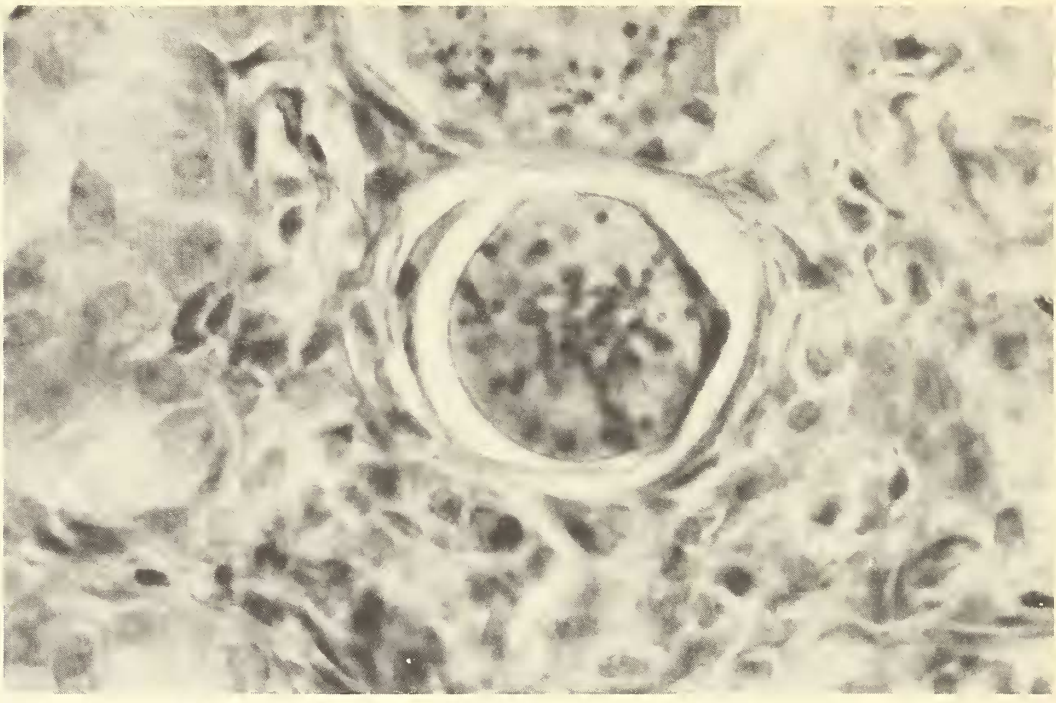

FIG. 19

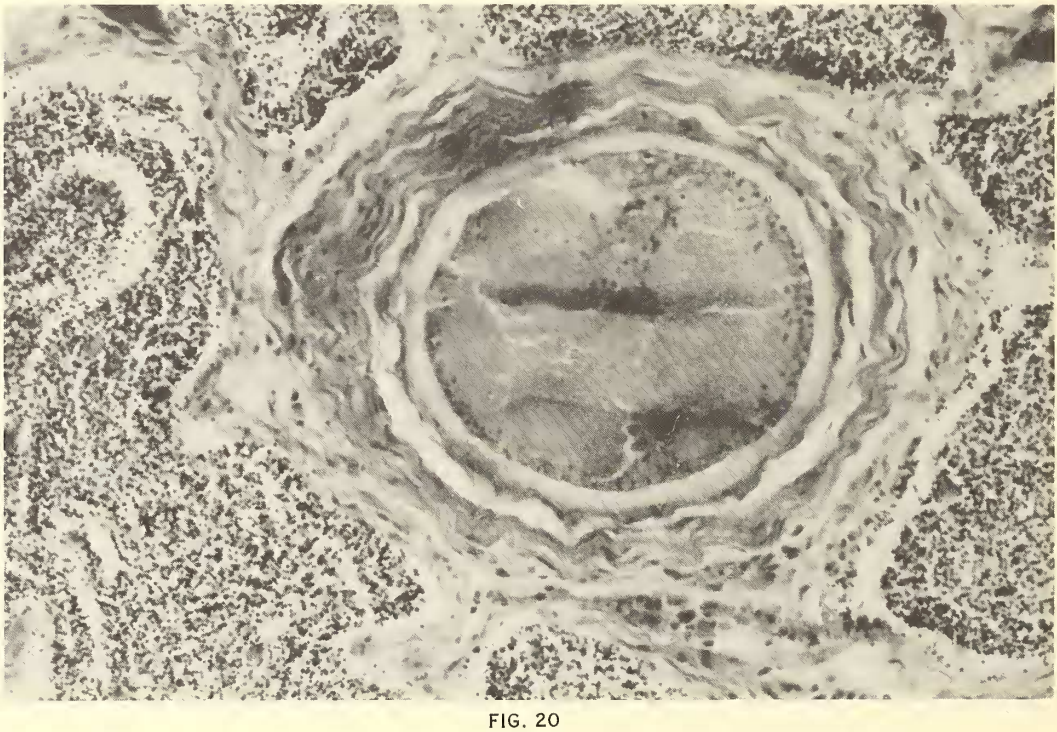

FIG. 20

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS